we are investigating the interaction between Cx32 and ZO-1 to identify if a common mechanism of binding exists with hDlg. These data provide new insights into the regulation of MAGUK family scaffolding proteins and Cx32 interactions.

1729-Pos Board B573
Recombinant Expression Screening of P. aeruginosa Bacterial Inner Membrane Proteins
Constance Jeffery, Vidya Madhavan.
University of Illinois, Chicago, IL, USA.
Transmembrane proteins (TM proteins) make up 25% of all proteins and play key roles in many diseases and physiological processes. However, much less is known about their structures and molecular mechanisms than for soluble proteins. Problems in expression, solubilization, purification, and crystallization cause bottlenecks in the characterization of TM proteins. This project addressed the need for improved methods for obtaining sufficient amounts of TM proteins for determining their structures and molecular mechanisms.
We obtained plasmid clones encoding eighty-seven transmembrane proteins with varying physical characteristics, for example, the number of predicted transmembrane helices, molecular weight, and grand average hydrophobicity (GRAVY). All the target proteins were from P. aeruginosa, a gram negative bacterial opportunistic pathogen that causes serious lung infections in people with cystic fibrosis. We measured the relative expression levels of the transmembrane proteins under several culture growth conditions. The use of E. coli strains, a T7 promoter, and a 6-histidine C-terminal affinity tag resulted in the expression of 58 out of 87 test proteins (68%). In this study, factors related to overall hydrophobicity and the number of predicted transmembrane helices correlated with the relative expression levels of the target proteins.
Identifying physical characteristics that correlate with protein expression might aid in selecting the "low hanging fruit", or proteins that can be expressed to sufficient levels using this sort of expression system. The use of other expression strategies or host species might be needed for sufficient levels of expression of transmembrane proteins with other physical characteristics. Surveys like this one could aid in overcoming the technical bottlenecks in working with TM proteins and could potentially aid in increasing the rate of structure determination.

1730-Pos Board B574
Molecular Determinants of Neisserial Pathogenesis: Mapping the Interaction Between Opa I and a Human Binding Partner CEACAM1
Christopher Reyes, Daniel A. Fox, Kalyani Jambunathan, Thien Nguyen, Izabela Bielnicka, Linda Columbus.
University of Virginia, Charlottesville, VA, USA.
Neisserial Opa proteins mediate the internalization of the bacterial cell by host epithelial cells via an interaction between the extracellular loops of Opa proteins and the extracellular domains of the host binding partner present on the cell surface. The eleven Opa proteins can be subdivided into two classes on the basis of the human receptor target. The Opa_{HS} class is named for heparan sulfate proteoglycan (HSPG), while Opa_{CEA} proteins bind carcinoembryonic-antigen related cell adhesion molecules (CEACAMs), of which there are seven varieties. Significantly, each of the Opa_{CEA} proteins has a characteristic specificity for each CEACAM. Of the four extracellular loops of Opa proteins, binding specificity is attributed primarily to two, which correspond to hypervariable regions of the protein sequence. However, mutational and chimeric analyses have not revealed the sequence determinants of the hypervariable regions that are responsible for receptor target recognition. Furthermore, it has been shown that the binding requires a cooperative interaction between the two hypervariable domains, and that specificity is determined by specific pairing of the sequences. It is the goal of this study to determine at a molecular level how specificity is attained by studying the structure and dynamics of the Opa I - receptor interactions. To this end, OpaI, which binds to CEACAM1 receptors, has been cloned, expressed, purified, and refolded and the NMR backbone assignment is in progress. The progress towards structure determination will be presented. In addition, NMR data mapping the interactions between Opa I and the soluble receptor will be presented in order to characterize the functionally relevant structural interactions involved in bacterial pathogenesis.

1731-Pos Board B575
Associate Professor of Chemistry
Garth J. Simpson.
Purdue University, West Lafayette, IN, USA.
Second-order nonlinear optical imaging of chiral crystals (SONICC) enables sensitive and selective detection of sub-diffraction limited protein microcrystals with negligible contributions from solvated proteins or amorphous protein aggregates. Under low magnificaiton (large field of view) applications, SONICC can detect individual crystals as small as 100 nm in diameter, which is conservatively 6 orders of magnitude lower that achievalbe using current methods for crystal detection in commercial screening platforms. In studies of microcrystallites of green fluorescent protein (GFP) prepared in 500 pL droplets using a crystallization micro-array, the SHG intensities rivaled those of fluorescence but with superb selectivity for crystalline regions. Furthermore, SONICC is directly compatibility with virtually all common protein crystallization platforms without modification.

1732-Pos Board B576
Expression, Reconstitution and Biophysical Studies of Neuronal Uncoupling Proteins: UCP4 and UCP5
Marina V. Ivanova[1], Matthew D. Smith[2], Masoud Jelokhani-Niaraki[1].
[1]Department of Chemistry, Wilfrid Laurier University, Waterloo, ON, Canada, [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON, Canada.
Uncoupling proteins (UCPs), located in the inner membrane of the mitochondria, uncouple ATP-synthesis from the respiratory chain by transporting protons across the inner membrane into the matrix, hence dissipating the proton-motive force and releasing heat. The neuronal UCPs (nUCPs), UCP4 and UCP5, were discovered recently (in 1998) and little is known about their structure and function. To gain further insight into the potential importance of these two proteins in the neuroprotection and neuromodulation of neurodegenerative diseases, this study will focus on the structure, function and interaction of the nUCPs with nucleotides (inhibitors) and fatty acids (activators). A recombinant version of the proteins, utilizing a hexa-histidine tag and a TEV protease site (for subsequent His-tag cleavage) has been designed, expressed as insoluble inclusion bodies, and isolated and purified using immobilized metal affinity chromatography. Subsequent reconstitution of the proteins in mild detergent (DDM and digitonin) allowed for biophysical studies by circular dichroism and fluorescence spectroscopy. Circular dichroism spectroscopy has shown that, similar to the recombinant UCP1, nUCPs possess dominantly helical structures in digitonin and DDM [1]. Furthermore, detergent-mediated reconstitution of the proteins into preformed liposomes can give more physiologically relevant structural and functional information. Comparison of the structure and function of human UCP1 (thermogenin) to nUCPs, in lipid membranes and membrane-like environments, will eventually show whether these proteins have any similarity in conformation and functional behaviour.
[1] Jelokhani-Niaraki, M., Ivanova, M.V., McIntyre, B.L., Newman, C.L., McSorley, F.R., Young, E.K. and Smith, M.D. (2008) *Biochem. J.*, **411**, 593-603.

1733-Pos Board B577
Helix-Helix Interactions in Membrane Proteins: Structural Analysis and Free Energy Calculations of Polyleucine-based Dimers
Anirban Mudi, D. Peter Tieleman.
University of Calgary, Calgary, AB, Canada.
According to Popot and Engelman's 'two stage' model, lateral interaction of helices in the membrane is a key step towards the folding of helical membrane proteins. In order to understand, how protein self-association regulates biological functions and how mutations may be involved in diseases, it is important to understand the constituent free energies. In this study, we investigated the effect of a wide range of mutations, at the 'a' or 'd' position of the heptad in the amino acid sequence, on the helix-helix interactions in a polyleucine dimer in lipid (DOPC) environment. We used molecular dynamics simulations and the thermodynamic integration method to calculate the free energies for various mutations. Our free energy calculations show that highly polar residues like Asn, Asp, Gln, Glu, Cys, and His enhance the stability of the polyleucine dimers much more than that by smaller polar residues like Ser and Thr, which agrees with previous experimental studies on similar peptides [1,2]. A past study showed that Ser, Thr, and less polar amino acids occur at a higher frequency, as compared to highly polar residues (Asn, Asp, Gln, Glu, Cys, His), in membrane proteins [2]. Recent experiments suggest that there is a high occurrence of weak H-bonds in membrane proteins [3]. Our findings and other studies suggest that membrane proteins prefer a wide range of moderately stabilizing interactions instead of strong ones, which lends them a greater degree of flexibility in terms of conformation and stability [3,4].
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1734-Pos Board B578
Assembling Within The Lipid Membrane: Viral Membrane Proteins
Wolfgang B. Fischer.
National Yang-Ming University, Taipei, Taiwan.
Viruses encode a number of membrane proteins which are interacting with lipid membranes. One class of these proteins are known to form homo-oligomeric